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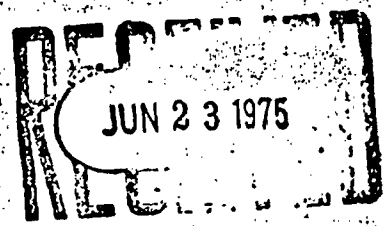
THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

Date:



1. Principal Investigator (give title and degrees):

James A. Will, DVM, Ph.D.
Professor and Chairman

2. Institution & address: Department of Veterinary Science
University of Wisconsin
1655 Linden Drive
Madison, WI 53706

3. Department(s) where research will be done or collaboration provided:

Department of Veterinary Science and Waisman Center

4. Short title of study: Morphologic and functional correlations of the APUD cells of the lung.

5. Proposed starting date: 1 January 1976

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

a) Define the function of the APUD cells of the lung.

b) Correlate the alterations in function of the lung with morphologic changes in these cells.

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8. *Brief statement of working hypothesis:*

Our working hypothesis and supporting evidence are as follows: The Feyrter cells and the NEBs (Neuroepithelial bodies)of the lung have been demonstrated by morphological means to meet the criteria for APUD cells as established by Pearse (1). Current literature supports the facts that these cells are similar to carotid body type I cells, that they are amine containing (2), that the granules within the cells can be depleted by reserpine (3), the fact that they may be modified by acute hypoxia (3,4) and that they have dual innervation and therefore perhaps act as both receptor and effector cells in the airway of the lung (3). By virtue of this evidence the hypothesis is presented that these cells are airway chemoreceptors and perhaps effectors that may influence the regulation of respiration, the control of broncho- and vaso-constriction, and therefore may directly or indirectly play an important role in the development, maintainance, and perhaps pathogenesis of pulmonary hypertension.

9. Details of experimental design and procedures (append extra pages as necessary)

Based on the above hypothesis the following questions can be asked and evidence obtained to support or reject the hypothesis:

1) Does the number and content of these cells remain constant in the change from neonate to the adult?

Moosavi, et al attempted to answer this question in a semi-quantitative way (4). These workers did not do a complete morphometric study. The fact that the number of Feyrter cells decreased per unit of bronchiolar epithelium did not take into consideration the growth of the lung during this 31 day period.

Experiment: We propose to use rabbits rather than rats. Pregnant females will be the adult subjects. Their young will be killed at intervals after birth and complete morphometric studies will be done to quantitate the numbers of these cells per unit of lung volume.

We propose to study the morphology and content of these cells by several techniques: Currently, one graduate student is working in Odense, Denmark with Dr. E. Hage (2) for 8 weeks to perfect techniques in (1) light microscopy and (2) formalin-induced-fluorescence (FIF). Dr. Hage has also agreed to come to this laboratory in September of this year to do a preliminary study with us. Professor Quay, a co-investigator has experience with (3) auto radiography and we plan to use these techniques in his laboratory with labelled amines, polypeptides, their precursors and metabolites. The content of the granules and the cells totally will be assessed by this technique as well as differential fluorescence. Finally, both Professor Quay and Professors Will and Bisgard jointly have postdoctoral fellows working on the (4) electron microscopy of these cells or similar cells. The combination of these four techniques should provide us with the ability to correlate changes in the cells and the granules within the cells with the functional studies we propose.

2) Is the effect of chronic hypoxia similar to that found acutely by Lauweryns, i.e. depletion of granular content consisting principally of serotonin?

Evidence in our own laboratory using methysergide and serotonin in aerosolized form implies that serotonin is not the principal mediator of hypoxic vasoconstriction (5). We used aerosolized compounds as well as those administered IV because of evidence supported by the work of Hauge (6) which makes it likely that the receptors involved in the pulmonary vasoconstrictor response are located closer to the airway than the capillaries. Our work does not exclude the possibility that serotonin may be one of several mediators in the response. Lauweryns supports this by the fact that more than one compound appeared to be present in the cells. We have further demonstrated that animals exposed to chronic hypoxia have an exaggerated response to acute hypoxia (7).

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This implies that the mechanism may be different for acute and chronic hypoxia. This is supported by work done by Tucker and Reeves(8). Our studies also show that this change in reactivity is not related entirely to muscular hypertrophy of the pulmonary vasculature, therefore the possibility exists that there is an alteration in lung metabolism initiated or mediated by substances elicited by these cells.

Experiment: A group of pregnant rabbits would be placed in the hypobaric chamber of the Biotron facility here at Madison or taken to our high altitude laboratory at Climax, Colorado (elevation 3400 m., P_{BP}=510 mmHg) and the first experimental protocol would be duplicated.

A further question that would be answered would be: Does exposure to chronic hypoxia cause the number of these cells to increase or have increased activity in the adult as well as the neonatal animal?

3) If these are chemoreceptors do they change in number or content when the carotid bodies and the aortic bodies are denervated?

Work in our laboratory in calves and ponies has demonstrated that animals with the carotid bodies removed and in the case of ponies, the aortic bodies also denervated tend to develop pulmonary hypertension at higher P_{IO_2}s because of the tendency to hypoventilate and develop low alveolar PO_2s (9,10).

Experiment: Although our experience is mainly with the carotid body removal and aortic body denervation in large animals,we propose to use the rabbit for the initial morphologic correlative studies because this species is unique in that it has a separate nerve to the aortic body which can be severed without thoracotomy.

Animals will be prepared and treated in groups similar to experiments 1 and 2.

Once the morphologic studies in experiments 1, 2, and 3 enforce or reject the postulates presented, studies will be extended to larger unanesthetized species like the dog and pony where work will be continuing on ventilatory control. Hopefully dual use can be made of the surgically prepared animals. These animals are much more adaptable to complete functional studies than the rabbit or other laboratory animals, particularly if the studies are performed in the unanesthetized state.

4) If these are chemoreceptors, can they be influenced by the administration of aerosolized compounds which will block, stimulate, deplete, poison, release, or otherwise modify the metabolism of these compounds contained in the granules of these cells?

We have preliminary evidence that low levels of an H_1 blocker that is administered by aerosol modified the vasoconstrictive response to acute hypoxia. This same blocker placed IV required toxic doses that could only be administered to anesthetized animals or in isolated perfused lungs to be effective.

Experiment: Actually this would be a long series of experiments which would hopefully pharmacologically characterize these cells functionally and using the morphological methods developed in earlier experiments to correlate the results. Agents such as α-Methyltyrosine, α and β adrenergic agents, cyanide, doxapram, lidocaine, ethanol, etc., could be used. Complete hemodynamic studies would be done including pressures, cardiac output, oxygen content, heart rate, blood gases, hematocrit, and hemoglobin. Resistances, oxygen transport, work indices, etc. would be calculated. Pulmonary parameters such as ventilation, ventilatory rate,volumes, alveolar gases, compliance can be measured and calculated.

It is obvious that the direction that these studies will go is dependent upon the success and findings of the initial studies.

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9. a. BIBLIOGRAPHY

1. Pearse, A.G.E.: The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic and pathologic implications of the concept. J. Histochem. Cytochem. 17: 303-313, 1969.

2. Hage, Esther: Amine-Handling Properties of APUD-cells in the Bronchial Epithelium of Human Foetuses and in the Epithelium of the Main Bronchi of Human Adults. Acta path. microbiol. scand Section A, Vol. 81: 64-70, 1973.

3. Lauweryns, J. M. and Marnix Cokelaere: Hypoxia-sensitive Neuro-epithelial Bodies; Intrapulmonary Secretory Neuroreceptors, Modulated by the CNS. Z. Zellforsch. 145: 521-540, 1973.

4. Moosavi, H., P. Smith, and D. Heath: The Feyrter Cell in Hypoxia. Thorax, Vol. 28: 729-741, 1973.

5. Ungerer, T., J. A. Orr, G. E. Bisgard and J. A. Will: Hemodynamic Responses in the Pig; The Acute Effects of Serotonin and Hypoxia. Abstract. Presented at 55th Conference of Research Workers in Animal Diseases. Dec. 2-3, 1974. Copy appended to application.

6. Hauge, A.: The pulmonary vasoconstrictor response to acute hypoxia. Prog. Resp. Res. 5: 145-155, 1970.

7. Ruiz, A. V., G. E. Bisgard, I. B. Tyson, R. F. Grover, and J. A. Will: Regional Lung Function in claves during acute and chronic pulmonary hypertension. J. Appl. Physiol., 37: 384-391, 1974.

8. Tucker, A. and J. T. Reeves: Non-sustained pulmonary vasoconstriction during acute hypoxia in anesthetized dogs. Am. J. Physiol. 228: 756-761, 1975.

9. Bisgard, G. E. and Vogel, J. H. K.: Hypoventilation and pulmonary hypertension in calves after carotid body excision. J. Appl. Physiol. 31: 431-438, 1971.

10. Forster, H. V., G. E. Bisgard, B. Rasmussen, D. D. Buss, J. A. Orr, and M. Manohar: The Effect of Peripheral Chemoreceptor Denervation on Ventilatory Acclimatization to Hypoxia in Ponies. Abstract. To be presented to APS Fall Meeting, Oct. 5-11, 1975. Copy appended to application.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

A complete cardiopulmonary laboratory is available under the direction of Professors Will and Bisgard. A departmental laboratory operates to provide histological tissue preparations. A tissue freeze drier was obtained last year. Electron microscopes are available in the Department of Veterinary Science, the Muscle Biology Institute and the Waisman Center.

Professors Bisgard and Will in cooperation with Dr. R. F. Grover, University of Colorado Medical Center have a research laboratory at Climax, Colorado. There are living quarters, a corral for large animals and a laboratory building. This facility provides an ideal situation for large animal studies that can't be accommodated in a hypobaric chamber or for small animal experiments that have too many animals to fit into the chamber or are of such a long term that it is too costly to do in the chamber.

Professor Quay has a laboratory equipped to do autoradiography.

Animal rooms are available for large and small animals.

11. Additional facilities required:

Funds were asked for animal cages. While rooms and animal care is available, there is a shortage of cage facilities for rabbits.

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12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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R: REDACTED MATERIAL

12. a.

BIOGRAPHICAL SKETCH

Name: James Arthur Will, D.V.M. Address: REDACTED
REDACTED
REDACTED

Born: REDACTED Birthplace: REDACTED

Citizenship: REDACTED Marital Status: REDACTED

EDUCATION:

High school graduated from: Wauwatosa High School Year: R

University Attended	Dates Attended	Degree	Date	Major and Minor
University of Wisconsin	REDACTED	B.S.	R	Agriculture Animal Husbandry
University of Wisconsin	REDACTED	M.S.	R	Animal Science Genetics
Kansas State University	REDACTED	D.V.M.	R	Veterinary Medicine Pathology
University of Wisconsin	REDACTED	Ph.D.	R	Veterinary Science Comp. Cardiology

MILITARY SERVICE:

Branch: Army of the United States
QMC, 1st Lt.

From: Feb. 1954 to Feb. 1956

POSITIONS:

1952-53 Research Assistant, Department of Meat and Animal Science, University of Wisconsin, Madison, Wisconsin.

1956-60 Research Assistant, Kansas State University, Manhattan, Kansas.

1960-61 Practicing Veterinarian, Columbus Veterinary Hospital, Columbus, Wisconsin.

1961-64 Practicing Veterinarian, Self-employed, Columbus, Wisconsin.

1964-67 Postdoctoral Research Fellowship, University of Wisconsin, Department of Medicine, Cardiovascular Research Laboratory and Department of Veterinary Science - NIH (Candidate for Ph.D.) (3 years) December, 1967.

1966 Consulting veterinarian, Avian Pathology.

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1967 Assistant Professor of Veterinary Science and Assistant Scientist, Cardiovascular Research Laboratory, Department of Medicine, Medical School.

1971-74 Associate Professor of Veterinary Science, College of Agricultural and Life Sciences and Department of Medicine, Medical School.

1974 to present Professor and Chairman of Veterinary Science, College of Agricultural and Life Sciences and Staff member, Cardiovascular Research Laboratory.

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12. a. (continued)

BIOGRAPHICAL SKETCH (continued)

1972-73 Special Fellow, NHLI, Department of Pathology, New Medical School, University of Liverpool, Liverpool, England.

PROFESSIONAL SOCIETIES:

REDACTED

REDACTED

REDACTED

REDACTED

HONORS RECEIVED:

NIH Postdoctoral Fellow. 1964-1967.

Burr Beach Award. 1967.

NIH Special Postdoctoral Fellow. 1972-1973.

SPECIAL APPOINTMENTS:

Member, World Health Organization Committee for meeting on "Primary Pulmonary Hypertension in Man." October 15-17, 1972, Geneva, Switzerland.

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12. b.

BIOGRAPHICAL SKETCH

Name: Gerald Edwin Bisgard Birth Date: REDACTED

Present Home Address: REDACTED Place of Birth: REDACTED

Education:

Denver Public Schools, Graduated South High School - REDACTED

REDACTED

College Education:

B.S. Biological Science, Colorado State University -

D.V.M. Veterinary Medicine, Colorado State University - REDACTED

M.S. Clinical Medicine, Purdue University - R

Ph.D. Veterinary Science (Physiology), University of Wisconsin REDACTED

College Honors:

Sigma Xi
Beta Beta Beta
Omicron Delta Kappa
Phi Zeta
Graduation "With Distinction" (D.V.M.)

Professional Societies:

REDACTED

REDACTED

REDACTED

Positions Held:

1962-1967	Instructor in Veterinary Clinics, Purdue University.
1967-1969	Assistant Professor in Veterinary Clinics, Purdue University.
1967-1968	On Leave of Absence from Purdue University for Special Fellowship in Research at Cardiovascular Pulmonary Laboratory, University of Colorado Medical Center.
1969-1971	Special Fellowship in Research and Ph.D. Program, University of Wisconsin, Cardiovascular Laboratory, Department of Medicine and Department of Veterinary Science.
1971-1974	Assistant Professor of Veterinary Science, University of Wisconsin
1974-	Presently Associate Professor of Veterinary Science, University of Wisconsin

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12. c

BIOGRAPHICAL SKETCH

Name: Wilbur Brooks Quay REDACTED Title: Professor in Waisman Center and Department of Zoology

Role in proposed project: Co-investigator

Birthdate: REDACTED Nationality: REDACTED

Education:

Institution	Degree	Year	Field
Harvard College	A.B. (magna)	R	Biology
University of Michigan	M.S., Ph.D.	R	Zoology
Netherlands Central Institute for Brain Research	Res. Fellow	R	Brain and Neuroendo. Res.

Honors:

Phi Beta Kappa - Harvard, 1950; Sigma Xi - University of Michigan, 1952; Horace H. Rackham Predoctoral Fellow - University of Michigan, 1951-52; Professorships in Miller Institute for Basic Research in Science - University of California, Berkeley, 1964-65, 1971-72; various lectureships.

Major Research Interest: Central nervous system and neuroendocrinology; regulatory mechanisms and chronobiology.

Research and Professional Experience:

Professor, Department of Zoology, and Waisman Center, University of Wisconsin, Madison, Wisconsin. June, 1973 - present; research and teaching (undergrad. and grad. courses, train predoctoral and post-doctoral students).

Professor, Department of Zo-logy, University of California, Berkeley, 1967-1973.

Fellow, Netherlands Organization for the Advancement of Pure Research, at Netherlands Central Institute for Brain Research, Amsterdam.

Associate Professor, Department of Zoology, University of California, Berkeley, 1961-1967.

Assistant Professor, Department of Zoology, University of California, Berkeley, 1956-1961.

Instructor, Department of Anatomy, University of Michigan Medical School, 1952-1956.

Teaching Fellow, University of Michigan, Ann Arbor, spring 1950-51.

Teaching Assistant, University of Michigan, Ann Arbor, fall 1950-51.

Research Assistant, University of Michigan, Ann Arbor, 1949-1950.

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13. a.

1. B. E. McKenzie, B. C. Easterday and J. A. Will. Light and Electron Microscopic Changes in the Myocardium of Influenza Infected Turkeys. Amer. J. Path., Vol. 69, No. 2, Nov. 1972, pp. 239-254.

2. A. V. Ruiz, G. E. Bisgard, I. B. Tyson, R. F. Grover, and J. A. Will: Regional Lung Function in Calves During Acute and Chronic Pulmonary Hypertension. J. Appl. Physiol., 37(1974): 384-391.

3. J. A. Will and J. M. Kay. Hypertensive Pulmonary Vascular Disease Associated with Papain Emphysema in Rats. Respiration 31 (1974): 208-220.

4. Allin, E. F., Miller, J. M., Rowe, G. G. and J. A. Will: Effects of Intraperitoneal Administration of Proprandol on the Mouse Heart: Histochemical and Electron Microscopic Observations. Am. J. Card. 33 (May 6, 1974): 639-642.

5. Bisgard, G. E., J. A. Will, I. B. Tyson, L. M. Dayton, R. R. Henderson and R. F. Grover: Distribution of regional lung function during mild exercise in residents of 3100 m. Res. Physiol. 22(1974): 369-379.

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Source: https://www.industrydocuments.ucsf.edu/docs/npdm0000

13. b.

1. Bisgard, G. E., A. V. Ruiz, R. F. Grover and J. A. Will. Ventilatory control in the Hereford calf. J. Appl. Physiol. 35:220-226, 1973.

2. Ruiz, A. V., G. E. Bisgard, and J. A. Will. Hemodynamic responses to hypoxia and hyperoxia in calves at sea level and altitude. Pflugers Arch (European J. Physiol), 344:275-286, 1973.

3. Bisgard, G. E., A. V. Ruiz, R. F. Grover and J. A. Will. Ventilatory acclimatization to 3400 meters altitude in the Hereford calf. Respiration Physiol. 21:271-296, 1974.

4. Bisgard, G. E., J. A. Orr and J. A. Will. Hypoxic pulmonary hypertension in the pony. Am. J. Vet. Res. 36, No. 1 (Jan., 1975): 49-52.

5. Rawlings, C. A., M. L. Birnbaum, and G. E. Bisgard. Static pulmonary compliance in ponies. J. Appl. Physiol., 38(4):657-660, 1975.

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Source: https://www.industrydocuments.ucsf.edu/docs/npdm0000

13. c.

1. 1974. (Quay, W. B.) Pineal canaliculi: demonstration, twenty-four-hour rhythmicity and experimental modification. American Journal of Anatomy. 139(1): 81-93.

2. 1974. (Quay, W. B.) Pineal Chemistry: In Cellular and Physiological Mechanisms. Charles C. Thomas Publisher, Springfield, Illinois, pp. i-xv, 1-430, 68 Figures, 91 Tables.

3. 1973. (Lew, Gloria M., and W. B. Quay) Circadian rhythms in catecholamines in organs of the golden hamster. American Journal of Physiology. 224: 503-508.

4. 1973. (Lew, Gloria, and W. B. Quay) The mechanism of circadian rhythms in brain and organ contents of norepinephrine: Circadian changes in the effects of methyltyrosine and 6-hydroxydopamine. Comparative and General Pharmacology. 4(16): 375-382.

5. 1972. (Quay, W. B.) Pineal vasoconstriction at daily onset of light: its physiological correlates and control. The Physiologist. 15(3): 241.

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14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
James A. Will, Principal Investigator	30	
Gerald E. Bisgard, Co-investigator	10	
Wilbur B. Quay, Co-investigator	5	
Aureliano Hernandez, Research Assistant	50	
Barbara A. Meyers, Research Assistant	50	
Technical		
Lab Tech II - To be recruited ($8,124 plus fringe benefits $2,063)	100	$10,187
Sub-Total for A		$10,187

B. Consumable supplies (by major categories)

Radioisotopes	1,500
Animals and animal care	1,500
Functional study supplies: Cardiogreen, recording paper, surgical supplies, etc.	1,500
Morphology study supplies: EM, auto radiography, FIF	1,500
Sub-Total for B	6,000

C. Other expenses (itemize)

Biotron rental of hypobaric chamber, 90 days @ $15.30/day	1,377
Meeting travel	1,000
Travel to high altitude laboratory and living expenses	1,000
Equipment repair	500
Publishing costs cnt	300
Sub-Total for C	4,177
Running Total of A + B + C	20,364

D. Permanent equipment (itemize)

Animal cages	1,000
Sub-Total for D	1,000
E	3,054
Total request	24,418

E. Indirect costs (15% of A+B+C)

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	10,798	7,500	5,500	1,000	3,570	28,368
Year 3	11,445	8,500	6,000	1,000	3,892	30,837

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16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. Chemoreceptors and Ventilatory Control (Bisgard and Will)	NIH, HL 15473	$69,000	9/1/73 to 8/31/76
2. Mechanisms of Pulmonary Hypertension (Bisgard and Will)	UW # 133-A280 Wis. Heart Ass.	7,500	7/1/75 to 6/30/76
3. Morphologic and Functional Correlations of the APUD Cells of the Lung (Will and Bisgard)	UW Grad. School # 160243	7,344	7/1/75 to 6/30/76
4. Circadian Phase Shifts and Mental Health (Quay)	NIH, MH 22305-01	30,000/yr	6/1/73 to 5/31/77

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to
Board of Regents of the University of Wisconsin System

Mailing address for checks
750 University Avenue
Madison, WI 53706

Principal investigator
Typed Name James A. Will
Signature James A. Will Date 11 June 75
Telephone (608) 262-3177 212
Area Code Number Extension

Responsible officer of institution
Typed Name Robert W. Erickson
Title Director, Research Administration-Financial
Signature Robert W. Erickson Date 6/19/75
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Area Code Number Extension

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